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<b>13. ABSTRACT (Maximum 200 Words)</b>  Androgen receptor (AR) plays a critical role in prostate oncogenesis. Allelic variants of AR, particularly in length of an N-terminal glutamine (Q) tract, are associated with distinct risks of disease. Mutation of AR in tumors may enter into resistance to treatment and androgen independence. Initiation and progression have been difficult to study due to lack of early disease samples and lack of animal models. This has been partly overcome with transgenic mouse tumor models. However, mouse AR differs significantly from human in the N-terminus. In order to critically evaluate the role of the polymorphic glutamine tract in disease, and to identify relevant sites in the N-terminus whose mutation can lead to androgen-independent AR function, we have humanized the mouse by converting its androgen receptor gene to the human sequence. We have done this by homologous recombination in embryonic stem cells, introducing AR alleles with 12, 21, or 41 glutamines. Mice bearing the wild type allele with 21 glutamines are normal by all indications, including microarray analysis. However, expression of specific androgen target genes suggests subtle distinctions exist. This will be explored in detail by examining tumor progression and by sequencing AR cDNAs from tumors of castrated vs. intact mice.				
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## INTRODUCTION

The development and progression of prostate cancer (PCa) depends on genetic and environmental factors that are still poorly defined. One thing all tumors have in common, however, is an initial dependence on androgen for growth [1]. Polymorphisms in androgen receptor (AR) may impact risk of disease, and somatic mutations may enter into progression and response to therapy [2-4]. Endocrine therapy is initially successful, but tumors ultimately become androgen-independent and resist further treatment. Despite this, AR levels in the tumor remain high and the AR signaling pathway appears intact, highlighting a continued role of AR in the disease process. AR molecular genetics offers clues to two crucial problems in PCa: **1) How do polymorphisms in AR lead to greater risk of disease? 2) How do somatic mutations in AR during tumor growth circumvent hormone ablation?** This project addresses these questions for the human receptor in a transgenic mouse prostate cancer model, allowing initiation, progression and treatment of disease to be integrated experimentally. Elucidating the mechanisms by which genetic variants alter AR action will validate their use as molecular markers in treatment, and, ultimately, may reveal novel targets for therapy.

## BODY

Our hypothesis is that **genetic variation in human AR affects the initiation and progression of prostate cancer**. Germline variation affects initiation or aggressivity of early disease, while somatic mutations drive androgen-independent growth. To confirm this hypothesis, our first major objective was to replace the mouse AR with human sequences to study their effects in the mouse in general and in a transgenic tumor model (Transgenic Adenocarcinoma of the Mouse Prostate, or TRAMP [5]). An implicit sub-hypothesis is that **AR species differences impact differential PCa susceptibility**. Since Q (glutamine) tract length enters into PCa risk, we proposed to compare varying length hAR alleles. We also plan to compare mutations arising in androgen-dependent vs. -independent disease, to identify sites correlating with AR function. Our aims underlying the Statement of Work are as follows:

**Aim I.** To study the role in PCa of polymorphisms in human *Ar*, *mAr* will be “humanized” by homologous recombination in embryonic stem cells, to create three *h/mAr* alleles differing in glutamine tract length (12Q, 21Q, 41Q). Differences in androgen action (fertility, behavior, molecular markers) and spontaneous prostate cancer will be studied in mice with *h/mAr* alleles.

**Aim II.** To determine the role of human AR variants on PCa initiation, *h/mAr* alleles will be placed on the TRAMP background for transgene-induced oncogenesis. Effect of the Q tract will be assessed on prostate pathophysiology and gene expression by cDNA microarray.

**Aim III.** To determine the role of AR variation on PCa progression, spontaneous mutations will be identified in AR cDNAs from castrated (androgen-independent) vs. intact *h/mAr*-TRAMP mice. The effect of mutations will be determined by introduction into ARs for transfection analysis, with and without coactivators. The effect of mutations on the oncogenic potential of prostate cells will be tested by tumor formation and metastasis in SCID mice.

As outlined, each Aim corresponds to a Task within the Statement of Work, and we are on schedule in accomplishing them. We have already "created" the 21 and 41 Q h./mAR mouse strains. ES cells with the 12 Q recombined allele have been reinjected into blastocysts, because the first set of chimeric mice failed to generate pups with the targeted allele. The 21 Q h./mAR mice have been in existence nearly 6 months; homozygous females as well as males show no differences in gross appearance, behavior, or fertility from littermates with the mouse AR gene. Mice of this lineage are being generated for aging studies and to track prostate physiology, which will occur over the next two years. These mice have also been crossed onto the TRAMP background, and at the age of ten weeks one cohort (ten mice) was castrated and another left intact. Tumors from these mice are being collected, either when the tumor becomes about one centimeter by palpation, or when the mice are 26-30 weeks of age. AR cDNAs will be analyzed from these tumors for mutations occurring with progression.

To characterize the 21Q h./mAR mice, we have examined gene expression profiles by cDNA microarray analysis. RNA was extracted from tissues of h./mAR or wild type control male mice, using Trizol Reagent. cDNA was reverse transcribed (CyScribe FS cDNA Kit) from pooled samples of three mice each (h./mAR or control), labeling one with Cy3 dye and the other with Cy5. Both probes were hybridized to the same slides overnight at 45 C. The slides contained a 5K Mouse Array, spotted by the University of Michigan Cancer Center and Prostate SPORE Microarray Facility, with about 2500 known genes and about 2500 ESTs, from the Research Genetics Mouse cDNA library. Slides were washed, dried and scanned using an Axon GenePix 4000 Scanner. Before analyzing the data, spot intensities were filtered to screen out low-expressing genes with high signal variability. The log<sub>2</sub> ratio of humanized to control mouse expression was calculated and differential gene expression between samples was assessed. We assessed initially testis, kidney and liver – in all three tissues nearly all genes showed equal expression in humanized and control mouse samples. This suggests that the human AR can substitute for the mouse AR without any gross changes in gene expression. A small number of genes in each tissue did show differential expression, particularly in testis. The microarray analysis will be repeated twice more to determine whether these differences are reproducible or reflect experimental or technical error.

While bulk gene expression appeared unaltered by substitution of human for mouse AR, we examined specific androgen target genes more directly by Northern blotting, in intact and castrated mice. In liver, the mouse major urinary protein (MUP) genes are regulated by androgen indirectly, as is the cytochrome P450 2d-9 (cyp 2d-9). Both these mRNAs were present at 2-fold higher levels in the humanized mice than in control mice. Furthermore, upon androgen withdrawal by castration, these mRNAs declined more slowly in the humanized mice. In kidney, ornithine decarboxylase (ODC) is a direct androgen target, and it too was expressed at higher levels in humanized than control mice. This supports the idea that the human AR N-terminus is stronger than the mouse in transactivating gene expression [6]. However, cyp 2d-9, which is more abundant in male than female mouse kidney was expressed at higher levels in the control mouse than in humanized mice. This may provide a unique opportunity to dissect species and tissue specific examples of sexually dimorphic gene expression.

In sum and in accord with our original plan, the first year of this project has largely been spent in establishing the mouse strains for analysis. These lines in fact function as predicted, in

being physiologically normal but having subtle phenotypes when hormonal effects are examined in greater detail. Thus we are encouraged that these mice will in fact be valuable for assessing the role of androgen receptor in prostate cancer initiation and progression, may provide better subjects for preclinical testing, and may lead to new treatments for disease.

## **KEY RESEARCH ACCOMPLISHMENTS**

- Correctly targeted ES cell lines were successfully incorporated into mouse blastocysts, and led to creation of mouse strains containing human AR sequences in place of mouse.
- Short (12 Q) and long (41 Q) glutamine tract alleles have been constructed and similarly placed in mice, but are behind the 21Q allele in analysis.
- Microarray analysis of the 21Q (average) allele reveals no gross overall differences between the "humanized" AR compared to normal mice.
- Northern blotting of androgen target gene expression in several tissues reveals distinctions in gene regulatory capacity of human vs. mouse AR.

## **REPORTABLE OUTCOMES**

We are currently preparing a manuscript detailing the creation and preliminary physiological characterization of the "humanized" AR mouse. The cDNA microarray expression data needs to be repeated and statistically analyzed prior to conclusion. Meanwhile, we have presented some of the data locally, as well as at the Second International Conference on Prostate Cancer Research, Iowa City, 10/12/02, and at the Interprostate SPORE Conference, San Francisco, 12/3/02. This mouse model will form the basis for other experiments proposed as a project with the renewal of the University of Michigan SPORE in Prostate Cancer.

## **CONCLUSIONS**

Mouse strains carrying human rather than mouse AR sequences have been constructed. This will allow direct testing of the role of AR glutamine tract variation in initiation of prostate cancer, which may help clarify contradictory results from epidemiological studies. Further, tumors initiated by transgenes in these mice will allow tracking the role of AR, and AR mutants, in resistance to antiandrogen therapy and androgen independent growth. The site of mutations in human AR sequence may lead to downstream interacting proteins that will be novel and more effective targets in new treatment strategies.

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**APPENDICES** - none